1: Biochimie 1995;77(1-2):30-9 Related Articles, NEW Books, LinkOut

Implications of a functional large ribosomal RNA with only three modified nucleotides.

Sirum-Connolly K, Peltier JM, Crain PF, McCloskey JA, Mason TL.

Department of Biochemistry and Molecular Biology, University of Massachusetts, Amherst 01003-4505, USA.

The sequence and structure of the peptidyl transferase region of large subunit ribosomal RNA is highly conserved and specific modified nucleotides could be important structural or functional elements in the catalytic center responsible for peptide bond formation. In fact, it has not been possible to reconstitute active E coli 50S subunits from in vitro transcripts of 23S rRNA and total 50S proteins. It is significant therefore, that the PET56 gene of yeast encodes an essential ribose methyltransferase that specifically modifies a universally conserved nucleotide, G2270, in the peptidyl transferase center of the mitochondrial large ribosomal RNA (21S). Since the loss of this modification in yeast mitochondrial 21S rRNA severely affects the assembly of 54S subunits, it is likely that the analogous 2'-O-methylguanosine at position 2251 (Gm2251) in E coli 23S rRNA is also required for the assembly of 50S subunits. Gm could be a critical structural determinant for the correct folding of the rRNA, the binding of one or more ribosomal proteins, or the interaction of the rRNA with tRNA. Previous work has shown that the mitochondrial large rRNAs are minimally modified relative to the E coli and eukaryotic cytoplasmic rRNAs. By direct chemical analysis using combined high performance liquid chromatography-mass spectrometry, the modification status of the yeast mitochondrial rRNAs was reexamined, revealing the presence of Gm, Um and pseudouridine (psi) in 21S rRNA. The Um was mapped to nucleotide 2791, which corresponds to the ribose methylated and universally conserved U2552 in E coli 23S rRNA, and the psi has been recently mapped to position 2819, which corresponds to psi 2580 in E coli 23S rRNA. The retention of Um and psi nucleotides in the peptidyl transferase center of the otherwise minimally modified mitochondrial rRNAs suggests that these modifications, like Gm2270, might be essential for ribosome assembly or function or both.

PMID: 7541254 [PubMed - indexed for MEDLINE]

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A paradigm for local conformational control of function in the ribosome: binding of ribosomal protein S19 to Escherichia coli 16S rRNA in the presence of S7 is required for methylation of m2G966 and blocks methylation of m5C967 by their respective methyltransferases.

Weitzmann C, Tumminia SJ, Boublik M, Ofengand J.

Roche Institute of Molecular Biology, Roche Research Center, Nutley, NJ 07110.

We have partially purified two 16S rRNA-specific methyltransferases, one of which forms m2G966 (m2G MT), while the other one makes m5C967 (m5C MT). The m2G MT uses unmethylated 30S subunits as a substrate, but not free unmethylated 16S rRNA, while the m5C MT functions reciprocally, using free rRNA but not 30S subunits (Negre, D., Weitzmann, C. and Ofengand, J. (1990) UCLA Symposium: Nucleic Acid Methylation (Alan Liss, New York), pp. 1-17). We have now determined the basis for this unusual inverse specificity at adjacent nucleotides. Binding of ribosomal proteins S7, S9, and S19 to unmodified 16S rRNA individually and in all possible combinations showed that S7 plus S19 were sufficient to block methylation by the m5C MT, while simultaneously inducing methylation by the m2G MT. A purified complex containing stoichiometric amounts of proteins S7, S9, and S19 bound to 16S rRNA was isolated and shown to possess the same methylation properties as 30S subunits, that is, the ability to be methylated by the m2G MT but not by the m5C MT. Since binding of S19 requires prior binding of S7, which had no effect on methylation when bound alone, we attribute the switch in methylase specificity solely to the presence of RNA-bound S19. Single-omission reconstitution of 30S subunits deficient in S19 resulted in particles that could not be efficiently methylated by either enzyme. Thus while binding of S19 is both necessary and sufficient to convert 16S rRNA into a substrate of the m2G MT, binding of either S19 alone or some other protein or combination of proteins to the 16S rRNA can abolish activity of the m5C MT. Binding of S19 to 16S rRNA is known to cause local conformational changes in the 960-975 stem-loop structure surrounding the two methylated nucleotides (Powers, T., Changchien, L.-M., Craven, G. and Noller, H.F. (1988) J. Mol. Biol. 200, 309-319). Our results show that the two ribosomal RNA MTs studied in this work are exquisitely sensitive to this small but nevertheless functionally important structural change.

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L6 ANSWER 1 OF 1 CAPLUS COPYRIGHT 2002 ACS
AN 2001:781168 CAPLUS
DN 135:341165
TI Assay for identification of a **test compound** binding to a target RNA using a **RNA-modifying enzyme**
IN Murchie, Alastair Iain Hamilton; Lentzen, Georg Friedrich
PA Ribotargets Limited, UK
SO PCT Int. Appl., 48 pp.
CODEN: PIXXD2
DT Patent
LA English
FAN.CNT 1

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE

PI WO 2001079543 A2 20011025 WO 2001-GB1778 20010419
W: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, TZ, UA, UG, US, UZ, VN, YU, ZA, ZW, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM
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PRAI GB 2000-9772 A 20000419
US 2000-198179 P 20000419

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L8 ANSWER 1 OF 25 CAPLUS COPYRIGHT 2002 ACS
TI Method and compositions for drug discovery
SO PCT Int. Appl., 51 pp.
CODEN: PIXXD2
IN Pidgeon, Charles
AB Methods are disclosed for screening **test compds.** to identify those compds. exhibiting a potential biol. activity. A drug-**binding** substrate formed or identified using a drug substance having a predetd. biol. activity is used to screen and identify **test compds.** likely to exhibit the predetd. biol. activity. The potential biol. active **test compds.** are identified by their specific **binding** to the drug-**binding** substrates as detected by any of a wide variety of techniques using labeled or unlabeled assay components. In one embodiment a monoclonal antibody raised against a drug substance is used as a drug-**binding** substrate to identify and isolate **test compds.** in a natural product ext. or a combinatorial chem. library. Preferably the monoclonal antibody is characterized by its ability to **bind** specifically to at least one other drug substance having the same or similar biol. activity as the drug substance against which it was raised. The invention finds use inter alia in drug discovery protocols, in toxicity profiling of drug substances and in assaying com. natural products.

L8 ANSWER 2 OF 25 CAPLUS COPYRIGHT 2002 ACS
TI Assay for identification of a **test compound** **binding** to a target RNA using a **RNA-modifying** **enzyme**
SO PCT Int. Appl., 48 pp.
CODEN: PIXXD2
IN Murchie, Alastair Iain Hamilton; Lentzen, Georg Friedrich
AB A method for detg. whether a **test compd. binds** to a target RNA, the method comprising the steps of: (a) contacting the **test compd.** with the target RNA and a **RNA** **modifying enzyme**; and (b) detecting the modification of the target RNA by the enzyme and comparing the amt. of modification detected to that of a std. Inhibition of enzymic modification of the RNA target indicates that the **test compd.** can **bind**

the target RNA and can thus exhibit antibiotic activity. Various concns. of the L11 ribosomal protein were incubated with the Escherichia coli mGAR (methylatable GTPase-activating region) of 23S rRNA, [3H]-S-adenosyl-methionine and tsr methyltransferase. Methylation was inhibited as L11 concn. increased.

L8 ANSWER 3 OF 25 CAPLUS COPYRIGHT 2002 ACS
TI Screening assay methods and systems using target pooling
SO PCT Int. Appl., 38 pp.
CODEN: PIXXD2
IN Farinas, Javier A.; Wada, H. Garrett
AB Methods, devices and systems for increasing the throughput of screening assays by pooling multiple target systems, which allow a library of different materials, e.g., **test compds**., to be screened against the pooled targets to det. whether any of the materials affect one or more of the target systems. In preferred aspects, functioning of individual target systems is identified by differences in phys., chem. and/or optical properties particular to the target system in a target pool. The responses of CHO cells expressing the M1 muscarinic receptor and labeled with Fluo-4 and of THP-1 cells labeled with Fura red to carbachol and to UTP were detd. using a microfluidic device having a channel structure and a fluorescent detector that analyzes the different fluorescence signals simultaneously.

L8 ANSWER 4 OF 25 CAPLUS COPYRIGHT 2002 ACS
TI Structure, regulation and therapeutic use of human tyrosine phosphatase-like enzyme
SO PCT Int. Appl., 85 pp.
CODEN: PIXXD2
IN Xiao, Yonghong
AB Amino acid and encoding nucleotide sequences of human tyrosine phosphatase-like **enzymes** are disclosed. Identification of **test compds**. that **bind** to tyrosine phosphatase-like enzyme is described. Identification of a **test compd**. which decreases tyrosine phosphatase-like enzyme gene expression is also reported. Human tyrosine phosphatase-like enzyme can be regulated to treat or prevent diseases such as rheumatoid arthritis and osteoarthritis and carcinomas of the intestine, bladder, prostate, breast, stomach, and brain.

L8 ANSWER 5 OF 25 CAPLUS COPYRIGHT 2002 ACS
TI Substrates and screening methods for transport proteins
SO PCT Int. Appl., 144 pp.
CODEN: PIXXD2
IN Dower, William J.; Gallop, Mark; Barrett, Ronald W.; Cundy, Kenneth C.; Chernov-Rogan, Tania
AB A variety of methods for assaying libraries of **test compds**. as ligands and/or substrates of transport proteins, including both carrier-type and receptor-type transport proteins, are provided. Both in vitro and in vivo screening methods are disclosed. Also provided are methods for screening DNA libraries to identify members that encode transport proteins. Pharmaceutical compns. including compds. identified via the screening methods are also provided. CHO K1 cells expressing PEPT1 transporter of human or rat were prepd. Fluorescent XP10486 was synthesized and used as PEPT1 substrate.

L8 ANSWER 6 OF 25 EMBASE COPYRIGHT 2002 ELSEVIER SCI. B.V.
TI Effects of the nuclear factor-.kappa.B inhibitors 2-hydroxy-4-

trifluoromethylbenzoic acid and aspirin on micturition in rats with normal and inflamed bladder.

SO Journal of Urology, (2001) 166/5 (1962-1968).
Refs: 31
ISSN: 0022-5347 CODEN: JOURAA

AU Velasco C.; Angelico P.; Guarneri L.; Leonardi A.; Clarke D.E.; Testa R.

AB Purpose: We examined the effects of intravenous administration of the 2 nuclear factor-.kappa.B inhibitors aspirin and 2-hydroxy-4-trifluoromethylbenzoic acid (HTB) on bladder filling and voiding in anesthetized and conscious rats. Materials and Methods: Disappearance of isovolumic bladder contractions after intravenous administration of different doses of aspirin and HTB in anesthetized, transurethrally catheterized rats was evaluated. Cystometry was performed in conscious rats during bladder infusion with saline or diluted acetic acid as well as in those with cyclophosphamide induced cystitis. Changes in bladder capacity and voiding pressure were evaluated after intravenous administration of **test compounds**. Results: Aspirin induced a dose dependent disappearance of isovolumic bladder contractions in anesthetized rats with an extrapolated dose of 2.1 mg./kg. inducing 10 minutes of bladder quiescence. HTB was practically inactive, inducing a dose independent block of 3 to 4 minutes after intravenous administration of 1 to 10 mg./kg. In conscious rats with a bladder infused with saline aspirin was poorly active on bladder capacity, inducing a 20% increase 60 minutes after intravenous administration of 30 and 100 mg./kg. In rats with a bladder infused with acetic acid aspirin was much more active when injected at the initiation of inflammation and after I hour of irritant infusion. In this latter situation aspirin increased bladder capacity up to 60% after intravenous administration of 30 and 100 mg./kg. Similar results were obtained in rats with cyclophosphamide induced cystitis in which the bladder was infused with saline. In these cystometrography models 30 mg./kg. HTB intravenously was completely inactive. Conclusions: The results show that HTB is devoid of significant effects on the micturition reflex in the absence or presence of bladder inflammation, suggesting that acute inhibition of nuclear factor-.kappa.B does not influence bladder urodynamics in rats. In contrast, aspirin, which is a cyclooxygenase and nuclear factor-.kappa.B inhibitor, was always effective, indicating the important role of cyclooxygenase **enzymes**.

L8 ANSWER 7 OF 25 MEDLINE

TI Isothermal titration calorimetry in drug discovery.

SO PROGRESS IN MEDICINAL CHEMISTRY, (2001) 38 309-76. Ref: 181
Journal code: 0376452. ISSN: 0079-6468.

AU Ward W H; Holdgate G A

AB Isothermal titration calorimetry (ITC) follows the heat change when a **test compound binds** to a target protein. It allows precise measurement of affinity. The method is direct, making interpretation facile, because there is no requirement for competing molecules. Titration in the presence of other ligands rapidly provides information on the mechanism of action of the **test compound**, identifying the intermolecular complexes that are relevant for structure-based design. Calorimetry allows measurement of stoichiometry and so evaluation of the proportion of the sample that is functional. ITC can characterize protein fragments and catalytically inactive mutant **enzymes**. It is the only technique which directly measures the enthalpy of **binding** (delta H degree). Interpretation of delta H degree and its temperature dependence (delta Cp) is usually qualitative, not quantitative. This is because of complicated

contributions from linked equilibria and a single change in structure giving modification of several physicochemical properties. Measured delta H degree values allow characterization of proton movement linked to the association of protein and ligand, giving information on the ionization of groups involved in **binding**. Biochemical systems characteristically exhibit enthalpy-entropy compensation where increased bonding is offset by an entropic penalty, reducing the magnitude of change in affinity. This also causes a lack of correlation between the free energy of **binding** (delta G degree) and delta H degree. When characterizing structure-activity relationships (SAR), most groups involved in **binding** can be detected as contributing to delta H degree, but not to affinity. Large enthalpy changes may reflect a modified **binding** mode, or protein conformation changes. Thus, delta H degree values may highlight a potential discontinuity in SAR, so that experimental structural data are likely to be particularly valuable in molecular design.

L8 ANSWER 8 OF 25 BIOSIS COPYRIGHT 2002 BIOLOGICAL ABSTRACTS INC.DUPLICATE 1
TI Predicting drug pharmacokinetics in humans from in vitro metabolism studies.
SO Biochemical Society Transactions, (May, 2001) Vol. 29, No. 2, pp. 135-139.
print.
ISSN: 0300-5127.
AU McGinnity, D. F. (1); Riley, R. J.
AB The pharmaceutical industry is committed to market safer drugs with fewer side effects, predictable pharmacokinetic properties and quantifiable drug-drug interactions. There is an increasing need to develop robust, enhanced-throughput in vitro assays, which accurately extrapolate to humans. The major drug metabolizing human hepatic cytochrome P450s (CYPs; CYP1A2, 2C9, 2C19, 2D6 and 3A4) have been co-expressed functionally in Escherichia coli with human NADPH-cytochrome P450 reductase and validated as surrogates to their counterparts in human liver microsomes (HLM) with respect to their kinetic and inhibition properties. Using these recombinant **enzymes**, fully automated in vitro assays to assess CYP inhibition and determine the enzymology of drug oxidation have been developed and validated. IC50 values determined for a series of **test compounds** in HLM and recombinant CYPs were similar (r2=0.9, P<0.001). There was a good correlation between the sum of individual CYP intrinsic clearance (Clint) and HLM Clint (r2=0.8, P<0.001) for ten prototypic substrates for which clearance was CYP-dependent. Several in vitro incubation milieu (e.g. CYPs, HLM, human hepatocytes) are routinely used and the level of non-specific **binding** was investigated with respect to effects on Km and Ki determinations. There were clear correlations between **binding** and lipophilicity (logD7.4) for a selection of bases (r2=0.98, P<0.001) and acids (r2=0.79, P<0.001) that may allow prediction of this property. Our laboratory has shown that recombinant **enzymes** are suitable for 'frontline' predictive human metabolism studies in early drug discovery.

L8 ANSWER 9 OF 25 CAPLUS COPYRIGHT 2002 ACS
TI Surrogate cell-based system and method for assaying the activity of hepatitis C virus NS3 protease
SO PCT Int. Appl., 70 pp.
CODEN: PIXXD2

IN Pellerin, Charles; Lamarre, Daniel
AB The present invention concerns the development of a cell-based assay system having improved sensitivity to HCV NS3 protease activity when compared to known assays, which is useful for screening **test compds**. capable of modulating (particularly inhibiting) HCV NS3 protease activity. This system provides a first construct comprising a transactivator domain joined downstream of the NS3-5 domains of HCV under the control of a non-cytopathic viral promoter system. A second construct is also provided that comprises a reporter gene under the control of an operator sensitive to the **binding** of the transactivator. The NS3-5 domains encodes the NS3 polyprotein which comprises: the NS3 protease, followed by the NS4A co-factor, the NS4B and NS5A proteins (including any deriv., variant or fragment thereof), terminated by the NS5B protein (including any deriv., variant or fragment thereof) sufficient to constitute a NS5A/5B cleavage site. The transactivator, when expressed and released from the polyprotein initiates transcription and expression of the reporter gene that is measurable.

L8 ANSWER 10 OF 25 CAPLUS COPYRIGHT 2002 ACS
TI Transition state **binding** assay
SO PCT Int. Appl., 14 pp.
CODEN: PIXXD2
IN Grothaus, Paul G.; Davis, Dana E.; O'Malley, Sean
AB A method to det. whether a **test compd**. modulates the activity of an enzyme that has a metalated active site, comprises: (a) providing said **test compd**. coupled to a solid support; (b) treating said solid support with the enzyme in metalated form; (c) detg. as a pos. result of (b) that the enzyme **binds** to said solid support and as a neg. result of (b) that said enzyme does not **bind** to said solid support; (d) treating said solid support with said enzyme in a nonmetallated form; (e) detg. as a pos. result of (d) that the enzyme **binds** to said solid support and as a neg. result of (d) that said enzyme does not **bind** to said solid support; (f) whereby a pos. result in (c) and a neg. result in (e) identifies said **test compd**. as a modulator of said activity is disclosed.

L8 ANSWER 11 OF 25 CAPLUS COPYRIGHT 2002 ACS
TI Drug screening assay for inhibitors of bacterial translation initiation factor 3 (IF3) using a chimeric chloramphenicol acetyltransferase gene
SO PCT Int. Appl., 66 pp.
CODEN: PIXXD2
IN Dammel, Carol S.; Watson, Julie C.; Hernandez, Victor J.
AB The invention is directed to a high-throughput screening system for identifying new antibacterial agents specifically targeted against an essential component of the bacterial translation app., IF3. The assay uses a reporter gene system in whole cells, and is based on the ability of IF3 to discriminate against translation initiation at the atypical start codon of the reporter gene. Provided is a pAUU-CAT reporter gene construct in which the chloramphenicol acetyltransferase (CAT) gene has its ATG initiator codon replaced with ATT. Preferably, CAT is from E. coli. Inhibitors of IF3 are expected to result in increased growth of specially-designed test cells due to higher levels of expression from the reporter gene. The invention further provides secondary assays which are used to further study the **test compds**. which produced pos. results in the screening assays of the invention. The invention also encompasses reporter gene constructs, test cells, and compds. identified by the methods of the invention.

L8 ANSWER 12 OF 25 BIOSIS COPYRIGHT 2002 BIOLOGICAL ABSTRACTS INC. DUPLICATE 2

TI Bis-benzimidazole anticancer agents: Targeting human tumour helicases.

SO Anti-Cancer Drug Design, (Feb., 1999) Vol. 14, No. 1, pp. 19-36. ISSN: 0266-9536.

AU Soderlind, Krista-June; Gorodetsky, Brian; Singh, Ashok K.; Bachur, Nicholas R.; Miller, Gerald G.; Lown, J. William (1)

AB Certain DNA minor groove **binding** agents, distamycin, netropsin, and a series of anticancer bis-benzimidazoles can block DNA helicase activity by **binding** to duplex DNA at specific base sequences. DNA helicases are crucial to cell DNA replication, transcription and repair because these **enzymes** separate double-stranded DNA, thereby preparing the strands for enzymatic manipulation. From our studies we have developed a hypothesis that focuses on cellular DNA helicase action as a mechanistic site where these minor groove **binders** can act. A crucial aspect for modulation of DNA activity by drugs is for specificity and selectivity. A series of DNA-interactive bis-benzimidazole analogues of Hoechst 33258 was also prepared to explore the potential for anticancer activity mediated for certain of the drugs via bioreductive activation by endogenous NADH or NADPH. The biological endpoints examined included intracellular distribution in euoxic and hypoxic conditions observed by fluorescence microscopy; relative efficacy as antimetabolites determined by the MTT (tetrazolium salt, 3-(4,5-dimethyl-thiazol-2-yl)-2,5-diphenyl tetrazolium bromide) assay in euoxic and hypoxic conditions; and relative inhibitory activities on human DNA helicase, as determined by degree of dissociation of GC B6486 DNA. The intracellular distribution was unique to each of the **test compounds**. Compounds V-93 and V-153, the respective semiquinone and quinone derivatives, demonstrated the predicted enhanced cytotoxicity and anti-helicase activities, supporting the concept that preferential **binding** of DNA at 5'-CG and TG sequences provides a novel approach to anticancer drug development.

L8 ANSWER 13 OF 25 CAPLUS COPYRIGHT 2002 ACS

TI High-throughput assay for enzyme inhibitors and receptor- and target-**binding** ligands

SO PCT Int. Appl., 37 pp. CODEN: PIXXD2

IN Burbaum, Jonathan J.; Chung, Thomas D. Y.; Kirk, Gregory L.; Inglese, James; Chelsky, Daniel

AB A homogeneous high-throughput assay is described which screens compds. for enzyme inhibition, or receptor or other target **binding**. Inhibition (or **binding**) by the library compds. causes a change in the amt. of an optically detectable label that is bound to suspendable cells or solid supports. The amts. of label bound to individual cells or solid supports are microscopically detd., and compared with the amt. of label that is not bound to individual cells or solid supports. The degree of inhibition or **binding** is detd. using this data. Confocal microscopy, and subsequent data anal., allow the assay to be carried out without any sepn. step, and provide for high throughput screening of very small assay vols. using very small amts. of **test compd**.

L8 ANSWER 14 OF 25 CAPLUS COPYRIGHT 2002 ACS
TI Methods for screening **test compounds** for inhibiting **binding** of a CD4-HIV-1 complex to HIV-1 second receptors
SO PCT Int. Appl., 18 pp.
CODEN: PIXXD2
IN Neurath, Alexander Robert; Jiang, Shibo; Debnath, Asim Kumar; Li, Yun-yao;
Strick, Nathan
AB A method for screening a **test compd.** for inhibiting the **binding** of a CD4-HIV-1 complex to HIV-1 second receptors comprises: (a) prepg. a magnetic ligand by mixing a magnetic, CD4-contg. substrate with HIV-1, (b) mixing the magnetic ligand from step (a) with a **test compd.**, (c) adding cells that express the HIV-1 second receptors to the mixt. from step (b), (d) sepg. cells with bound magnetic ligands from cells without bound magnetic ligands by contact with a magnetic separator, and (e) quantifying the cells with bound magnetic ligands and quantifying the cells without bound magnetic ligands. The .beta.-chemokines RANTES, MIP-1.alpha., and MIP-1.beta. inhibited interaction of magnetic CD4-HIV-1 complexes with PM-1 cells expressing CD4 and receptors for RANTES/MIP-1.alpha./MIP-1.beta.. The cells were quantitated using the CyQUANT Assay Kit and the CytoFluor 2350 Fluorescence Measurement System (Millipore).

L8 ANSWER 15 OF 25 CAPLUS COPYRIGHT 2002 ACS
TI An affinity **binding**-based system for detecting particulates in a fluid
SO PCT Int. Appl., 92 pp.
CODEN: PIXXD2
IN Sheppard, Norman F., Jr.; Mian, Alec; Kellogg, Gregory; Kieffer-Higgins, Stephen G.; Carvalho, Bruce L.
AB This invention provides methods and app. for detecting and quantifying particulate matter suspended in a fluid. Specifically, the invention provides an integrated, affinity-**binding** based, anal. system comprising a platform for performing an affinity-**binding** based assay for specifically **binding** particulates including microbial cells, and a detection means for detecting the particulates specifically bound to a defined surface or chamber comprising the platform. Methods for using the anal. systems of the invention are also provided.

L8 ANSWER 16 OF 25 BIOSIS COPYRIGHT 2002 BIOLOGICAL ABSTRACTS INC. DUPLICATE 3
TI Bacterial complementation as a means to test enzyme-ligand interactions.
SO Applied Microbiology and Biotechnology, (Aug., 1998) Vol. 50, No. 2, pp. 181-186.
ISSN: 0175-7598.
AU Canyuk, B.; Craig, S. P., III; Eakin, A. E. (1)
AB A bacterial complementation assay has been developed for the rapid screening of a large number of compounds to identify those that inhibit an enzyme target for structure-based inhibitor design. The target enzyme is the hypoxanthine phosphoribosyltransferase (HPRT). This enzyme has been proposed as a potential target for inhibitors that may be developed into drugs for the treatment of diseases caused by several parasites. The screening assay utilizes genetically deficient bacteria complemented by active, recombinant enzyme grown in selective medium in microtiter plates.
By comparing absorbance measurements of bacteria grown in the presence and

absence of **test compounds**, the effect of the compounds on bacterial growth can be rapidly assayed. IC50 values for inhibition of bacterial growth are a reflection of the ability of the compounds to **bind** and/or inhibit the recombinant enzyme. We have tested this bacterial complementation screening assay using recombinant HPRT from the parasites Plasmodium falciparum and Trypanosoma cruzi, as well as the human enzyme. The results of these studies demonstrate that a screening assay using bacterial complement selection can be used to identify compounds that target **enzymes** and can become an important part of structure-based drug design efforts.

L8 ANSWER 17 OF 25 MEDLINE
TI DNA cleavage by topoisomerase I in the presence of indolocarbazole derivatives of rebeccamycin.
SO BIOCHEMISTRY, (1997 Apr 1) 36 (13) 3917-29.
Journal code: A0G; 0370623. ISSN: 0006-2960.
AU Bailly C; Riou J F; Colson P; Houssier C; Rodrigues-Pereira E; Prudhomme M
AB DNA topoisomerase I has been shown to be an important therapeutic target in cancer chemotherapy for the camptothecins as well as for indolocarbazole antibiotics such as BE-13793C and its synthetic derivatives NB-506 and ED-110 [Yoshinari et al. (1993) Cancer Res. 53, 490-494]. To investigate the mechanism of topoisomerase I inhibition by indolocarbazoles, we have studied the induction of DNA cleavage by purified mammalian topoisomerase I mediated by the antitumor antibiotic rebeccamycin and a series of 20 indolocarbazole derivatives. The compounds tested bear (i) various functional groups on the non-indolic moiety (X = CO, CH2, CHOH), (ii) a hydrogen or a chlorine atom at positions 1 and 11 (R2), and (iii) different substituents on the maleimido function (R1 = H, OH, NH2, NHCHO). Half of the ligands have the same carbohydrate moiety as rebeccamycin whereas the other ligands have no sugar residue. The inhibitory potency of the **test compounds** was assessed in vitro by comparing the cleavage of [32P]-labeled restriction fragments by the enzyme in the absence and presence of the drug. In addition, the DNA-**binding** properties of these compounds were investigated by means of complementary spectroscopic techniques including electric linear dichroism, and the DNA sequence selectivity was probed by DNase I footprinting. The study shows that the sugar residue attached to the indolocarbazole chromophore is critical for the drug ability to interfere with topoisomerase I as well as for the formation of intercalation complexes. Structure-activity relationships indicate that the presence of chlorine atoms significantly reduces the effects on topoisomerase I whereas the substituents on the maleimido function and the functional group on the non-indolic moiety can be varied without reduction of activity. The results suggest that the inhibition of topoisomerase I by indolocarbazoles arises in part from their ability to interact with DNA. Analysis of the base preferences around topoisomerase I cleavage sites in various restriction fragments indicated that, in a manner similar to camptothecin, the rebeccamycin analogue R-3 stabilized topoisomerase I preferentially at sites having a T and a G on the 5' and 3' sides of the cleaved bond, respectively. By analogy with models previously proposed for camptothecin and numerous topoisomerase II inhibitors which intercalate into DNA, a stacking model for the interaction between DNA, topoisomerase I and indolocarbazoles is proposed. These findings provide guidance for the development of new topoisomerase I-targeted antitumor indolocarbazole derivatives.

L8 ANSWER 18 OF 25 BIOSIS COPYRIGHT 2002 BIOLOGICAL ABSTRACTS INC.
TI Estrogenicity, antiestrogenicity and estrone sulfatase inhibition of

estrone-3-amine and estrone-3-thiol.
SO Journal of Steroid Biochemistry and Molecular Biology, (1995) Vol. 52, No.
3, pp. 281-286.
ISSN: 0960-0760.
AU Selcer, Kyle W. (1); Li, Pui-Kai
AB Estrogen levels in breast tumors of post-menopausal women are at least 10 times higher than estrogen levels in plasma. The high level of estrogen in
these tumors is postulated to be due to in situ formation of estrogen, possibly through conversion of estrone sulfate to estrone by the enzyme estrone sulfatase. Thus, inhibitors of estrone sulfatase are potential agents for the treatment of hormone-dependent breast cancers. We designed and synthesized a series of estra-1,3,5(10)triene-17-one, 3-amino and estra-1,3,5(10)triene-17-one, 3-thio derivatives. We have shown previously
that several of these compounds substantially inhibit estrone sulfatase, exceeding Danazol in their inhibitory activity. However, little is known about the metabolism of these compounds and the possible effects of their metabolites in vivo. Two probable metabolites of the synthetic estrone analogs are estra-1,3,5(10)triene-17-one, 3-amine (E-1-NH-2), and estra-1,3,5(10)triene-17-one, 3-thiol (E-1-SH). We tested these two compounds for estrogenicity, antiestrogenicity and inhibition of estrone sulfatase activity using a combination of in vivo and in vitro assays. The
ovariectomized rat uterine weight gain assay was used to test for estrogenicity. Neither E-1-NH-2 nor E-1-SH were estrogenic, as indicated by a lack of uterine weight gain when given at 25 mu-g/day for 7 days. The
test compounds also were not antiestrogenic, in that
they did not block estrone-induced uterine weight gain when given (100 mu-g/day) simultaneously with estrone (2 mu-g/day). Both compounds showed low affinity for the estrogen receptor. Using rat uterine cytosol as a source of estrogen receptor, the compounds displaced only a small percentage of (3H)estradiol **binding**, even when present at
1000-fold excess. Inhibition of estrone sulfatase activity was tested using human placental microsomes as a source of estrone sulfatase. E-1-NH-2 and E-1SH showed very low levels of estrone sulfatase inhibition (15.1 and 9.8%, respectively) under conditions where Danazol showed more than 60% inhibition. Our results indicate that neither of these two compounds would present significant problems if they were the primary metabolite in a treatment involving estrone sulfatase inhibition of estrogen-dependent breast cancer.

L8 ANSWER 19 OF 25 MEDLINE DUPLICATE 4
TI Flexible matching of test ligands to a 3D pharmacophore using a molecular superposition force field: comparison of predicted and experimental conformations of inhibitors of three **enzymes**.
SO JOURNAL OF COMPUTER-AIDED MOLECULAR DESIGN, (1995 Jun) 9 (3) 237-50.
Journal code: JCB; 8710425. ISSN: 0920-654X.
AU McMartin C; Bohacek R S
AB A computer procedure TFIT, which uses a molecular superposition force field to flexibly match **test compounds** to a 3D
pharmacophore, was evaluated to find out whether it could reliably predict
the bioactive conformations of flexible ligands. The program superposition
force field optimizes the overlap of those atoms of the test ligand and template that are of similar chemical type, by applying an attractive force between atoms of the test ligand and template which are close together and of similar type (hydrogen bonding, charge, hydrophobicity). A

procedure involving Monte Carlo torsion perturbations, followed by torsional energy minimization, is used to find conformations of the test ligand which cominimize the internal energy of the ligand and the superposition energy of ligand and template. The procedure was tested by applying it to a series of flexible ligands for which the bioactive conformation was known experimentally. The 15 molecules tested were inhibitors of thermolysin, HIV-1 protease or endothiapepsin for which X-ray structures of the bioactive conformation were available. For each enzyme, one of the molecules served as a template and the others, after being conformationally randomized, were fitted. The fitted conformation was then compared to the known **binding** geometry. The matching procedure was successful in predicting the bioactive conformations of many of the structures tested. Significant deviation from experimental results was found only for parts of molecules where it was readily apparent that the template did not contain sufficient information to accurately determine the bioactive conformation.

L8 ANSWER 20 OF 25 CAPLUS COPYRIGHT 2002 ACS
TI Bromopropylate: induction of hepatic cytochromes P450 and absence of covalent **binding** to DNA in mouse liver
SO Toxicol. Appl. Pharmacol. (1994), 129(1), 155-62
CODEN: TXAPA9; ISSN: 0041-008X
AU Thomas, Helmut; Sagelsdorff, Peter; Molitor, Elvira; Skripsky, Thomas; Waechter, Felix
AB Oral administration of benzilic acid ester-based acaricide bromopropylate at daily doses of 3, 15, 100, and 300 mg/kg body wt to young adult male Tif:MAGf mice for 14 days caused slightly increased liver wts. in the high-dose group. A dose-dependent increase of the microsomal cytochrome P 450 content was accompanied by elevated ethoxycoumarin O-deethylase, ethoxyresorufin O-deethylase, pentoxyresorufin O-depentylase, and total testosterone hydroxylase activities. When compared with mice treated in parallel with the model compds. for hepatic xenobiotic metabolizing enzyme induction, phenobarbitone, and 3-methylcholanthrene, the enzyme activity changes obsd. with bromopropylate largely equalled those expressed in phenobarbitone-treated mice. Immunochem. studies with monoclonal antibodies against rat liver cytochrome P 450 isoenzymes of the gene families 1A, 2B, 3A, and 4A confirmed that bromopropylate is a phenobarbitone-type inducer in the mouse liver. Titrn. of liver microsomal suspensions with bromopropylate yielded Type I substrate **binding** spectra. The specific amplitude was increased 1.5-fold when microsomes from bromopropylate-treated mice (300 mg/kg body wt) were used instead of control microsomes, indicating the induction of cytochromes P 450 catalyzing the oxidative metab. of the **test compd**. Single oral administration of 300 mg/kg body wt [14C]-bromopropylate to male mice, without or following pretreatment for 14 days with 300 mg/kg body wt unlabeled bromopropylate, gave no indication for DNA **binding** of the **test compd** . in the liver. This excludes a genotoxic potential via covalent DNA modification. The results suggest that, in analogy to phenobarbitone, bromopropylate acts as a tumor promotor rather than a tumor initiator in the mouse liver.

L8 ANSWER 21 OF 25 BIOSIS COPYRIGHT 2002 BIOLOGICAL ABSTRACTS INC. DUPLICATE 5
TI Drug-metabolizing **enzymes** in ligand-modulated transcription.
SO Biochemical Pharmacology, (1994) Vol. 47, No. 1, pp. 25-37.
ISSN: 0006-2952.

AU Nebert, Daniel W.

AB Genes encoding many of the so-called drug-metabolizing **enzymes** (DMES) are present in both prokaryotes and eukaryotes, suggesting that these genes arose on this planet more than 3.5 billion years ago-long before animal-plant divergence (estimated to be about 1.2 billion years ago) and long before the use and commercial development of drugs. What, therefore, are the real functions of DMEs? Several years ago I proposed that DMEs are upstream in the regulatory cascade of numerous signal transduction pathways, i.e. necessary for maintaining physiologically "safe", or "acceptable", steady-state levels of all small non-protein endogenous ligands (M-r = 250 +- 200) in each cell. Innumerable foreign chemicals and drugs mimic these small endogenous ligands, thus **binding** to a particular receptor and acting either as an agonist or antagonist in activating or inhibiting genes effecting growth, differentiation, apoptosis, homeostasis and neuroendocrine functions. Discussed in this review are additional examples consistent with this theory and not described in previous reviews, including: (i) insect-plant symbiosis; (ii) "cross-talk" amongst genes in the aromatic hydrocarbon-responsive (Ah) battery; (iii) signal transduction pathways involving the arachidonic acid cascade; and (iv) the explanation in carcinogen-screening studies as to why a maximum, or half maximum, tolerated dose (MTD, MTD-50) of many **test compounds** might cause cell division and tumorigenesis in experimental animals.

L8 ANSWER 22 OF 25 MEDLINE DUPLICATE 6

TI The role of receptor **binding** in drug discovery.

SO JOURNAL OF NATURAL PRODUCTS, (1993 Apr) 56 (4) 441-55. Ref: 26
Journal code: JA4; 7906882. ISSN: 0163-3864.

AU Sweetnam P M; Caldwell L; Lancaster J; Bauer C Jr; McMillan B; Kinnier W J; Price C H

AB Radioligand receptor **binding** has been used extensively to identify and characterize a host of receptors and **enzymes** targeting virtually every therapeutic area. Many drug discovery programs have been based on the utilization of radioligand receptor **binding** technology to identify lead compounds which interact with receptors likely to be important in neuronal, immunological, gastrointestinal, and cardiovascular function/dysfunction. There are several obvious advantages to using in vitro receptor **binding** as a first level screen when compared to in vivo pharmacometric screens. Scientifically, the structure activity data generated in **binding** assays is a direct reflection of the ligand/receptor interaction minus the complications which result from secondary events, bioavailability, and pharmacodynamic issues. Technically, the **binding** studies require only a small amount of **test compound** (< or = 1 mg), while whole animal studies routinely need gram quantities. Similarly, only a small amount of tissue is required, compared with the cost of purchase and maintenance of live animals for in vivo screening. Supply and labor costs are drastically reduced due to the limited volume and test tube based technology of receptor **binding**. For these reasons receptor **binding** assays have been utilized with considerable success to discover site specific lead compounds in virtually every therapeutic area.

L8 ANSWER 23 OF 25 EMBASE COPYRIGHT 2002 ELSEVIER SCI. B.V.

TI [Mucus models for investigation of intestinal absorption. Part: Validation and optimization].
MUCUSMODELLE ZUR UNTERSUCHUNG VON INTESTINALEN ABSORPTIONSMECHANISMEN. 1. MITTEILUNG: VALIDIERUNG UND OPTIMIERUNG DER MODELLE.

SO Pharmazie, (1992) 47/7 (505-515).
ISSN: 0031-7144 CODEN: PHARAT

AU Matthes I.; Nimmerfall F.; Sucker H.
AB With an in vitro retention and diffusion model we investigated a single step in the intestinal absorption of some drugs, i.e., the penetration through the outer continuous mucus layer overlying the enterocyte. The retention model was set up to investigate the interaction between drug and diluted mucus at equilibrium. Using native mucus the in vitro kinetics of penetration through the mucus layer was measured with the diffusion model and correlated with the in vivo data of intestinal absorption. The first part describes optimization procedures to select the most suitable way for preparation, the model system and the analytical methods and validation for mucus characterization. The decision to use duodenal mucus from pig instead of rat was based on the similarity with human mucus and the availability of a large amount of individual material compared to rat. The splitting of mucus to a water-soluble and insoluble fraction resulted only in quantitative, but not qualitative differences in retention of the tested compounds. Therefore, preparation of two fractions cannot be recommended. Lyophilized whole mucus reconstituted with water cannot be used in the diffusion model and in the other model, retention is lower compared to native mucus frozen at -20.degree.C. For retention and diffusion experiments native frozen mucus is the optimal preparation which can be used up to 6 month if stored at -20.degree.C. A small amount of DNA and microvilli were found as impurities in the mucus preparations. The hydrolytic activity of intestinal pancreatic **enzymes** was diminished during isolation of mucus. The reproducibility of analytical methods within and between batches, given as a percentage, was 2.4% and 14.3% for protein, 6.1% and 10.7% for DNA, 4.6% and 29.8% for total hexoses, 10.9% and 13.5% for glycoprotein, respectively. Changes in mucus consistency could be avoided with short incubation times because of proper area-volume ratio of the test cells, e.g. 4 h for retention and up to 1 h for diffusion experiments to meet sink conditions. The reproducibility of measurements with both models given as standard error was about 10%. The second part of this communication will deal with investigations on specific **binding** of **test compounds** to mucus using the validated retention and diffusion model described herein.

L8 ANSWER 24 OF 25 BIOSIS COPYRIGHT 2002 BIOLOGICAL ABSTRACTS INC.DUPLICATE 7
TI THE EFFECT OF SOME H-2-RECEPTOR ANTAGONISTS ON RAT HEPATIC MICROSOMAL CYTOCHROME P-450 AND LIPID PEROXIDATION IN-VITRO.
SO EUR J MED CHEM, (1989) 24 (1), 43-47.
CODEN: EJMCA5. ISSN: 0223-5234.
AU REKKA E; KOLSTEE J; TIMMERMAN H; BAST A
AB The H2-receptor antagonists 2-guanidino-4(3-methylaminomethyleneiminopheny l)thiazole DA 4643 and 2-methyl-4(4-isopropylaminomethyleneiminophenyl)imi dazole DA 5047 were studied for their interaction with cytochrome P-450 and for their influence on lipid peroxidation in vitro. Their activities were compared to those of cimetidine, ranitidine and tiotidine. The interaction with cytochrome P-450 was investigated by **binding** studies and determination of the influence on the mono-oxygenase and oxidase activities of cytochrome P-450. The effect of the **test compounds** on enzymatic (NADPH-induced) and non-enzymatic

(ascorbate-induced) lipid peroxidation was estimated using normal and heat-inactivated microsomes. The results indicate that DA 5047 and DA 4643 are weaker inhibitors of cytochrome P-450 compared to cimetidine tiotidine. Cimetidine, ranitidine, tiotidine and DA 5047 did not have any considerable effect on lipid peroxidation. Compo DA 4643 inhibited both enzymatic and non-enzymatic lipid peroxidation. This activity was apparently not related to inhibit of drug metabolizing **enzymes** but it is probably due to the anti-oxidant properties of this compound.

L8 ANSWER 25 OF 25 BIOSIS COPYRIGHT 2002 BIOLOGICAL ABSTRACTS INC. DUPLICATE 8

TI EFFECT OF BENZYDAMINE ON EXOCYTOSIS AND RESPIRATORY BURST IN HUMAN NEUTROPHILS AND MONONUCLEAR PHAGOCYTES.

SO AGENTS ACTIONS, (1985) 16 (5), 346-352.
CODEN: AGACBH. ISSN: 0065-4299.

AU BAGGIOLINI M; MAUDERLI P; NAF U; CATANESE B

AB The effect of benzydamine on stimulus-dependent respiratory burst activity and enzyme release was tested in human neutrophils, monocytes and monocyte-derived macrophages. Established anti-inflammatory compounds, indomethacin, phenylbutazone and bufexamac, were tested for comparison. Care was taken to avoid cytotoxic or cytolytic concentrations of the **test compounds**, and their effect on release of lactate dehydrogenase was also tested. Release of specific and azurophil granules contents were induced in human neutrophils by A23187, PMA and fMLP with and without cytochalasin B pretreatment. Benzydamine inhibited stimulus-dependent release of vitamin B12-**binding** proteins, a marker for the specific granules, in a concentration-dependent fashion. By contrast, phenylbutazone and bufexamac were practically inactive. The effect of benzydamine on exocytosis of azurophil granules was tested in cytochalasin B-pretreated neutrophils. Benzydamine, again in contrast to the two reference anti-inflammatory compounds, inhibited release concentration-dependently also under these condtions. The concentration-dependently also under these conditions. The concentration of the compound which inhibited exocytosis by 50% was 30-100 .mu.M in normal and 3-10 .mu.M in cytochalasin B-treated neutrophils. The effect of benzydamine and reference compounds on the respiratory burst was tested by assaying for superoxide formation in neutrophils and H2O2 formation in mononuclear phagocytes. Benzydamine was inactive on neutrophils and inhibited slightly the burst response of monocytes and macrophages. Two reference compounds, bufexamac and phenylbutazone, were generally more active. The strongest inhibitory effect was that of phenylbutazone on fMLP-stimulated cells. Benzydamine lacked activity under these conditions, indicating that it does not **bind** to the receptor of formylated chemotactic peptides. The profile of activity of benzydamine shown in these experiments on human phagocytes suggest that this compound may act therapeutically by decreasing the release of **enzymes** and other granule constituents from stimulated neutrophils.

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